

BIOCHEMISTRY 101  
AUTUMN QUARTER 1961 - 67

In recognition of and in the spirit of the Honor Code, I certify that I will neither receive or give unpermitted aid and that I will report to the best of my ability all Honor Code violations observed by me.

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Questions 1 through 7:

An infant was recently referred to the Palo Alto-Stanford Hospital with a diagnosis of possible glycogen storage disease of the von Gierke type. Following the preliminary examination and laboratory work-up, the case was presented at one of the weekly pediatric conferences. To begin with, a comparison was made between the characteristics of classical von Gierke's disease and those found in the present case; these are summarized in the accompanying table. The opinion of those present at the conference was unanimous; although this infant displayed massive deposition of glycogen and a marked enlargement of the liver, there was sufficient reason to doubt the original diagnosis. Moreover, there was general agreement that a correct diagnosis required measurements of the level of various enzymes involved in glycogen synthesis and utilization together with an analysis of the structure of the liver glycogen. Mr. Harbandray, one of the first-year medical students who was present at the conference, saw in this an opportunity for an interesting research problem and he volunteered to obtain this information.

1. To confirm the tentative conclusion that this infant did not have classical von Gierke's disease, he obtained a liver sample by biopsy and tested for (choose the one which is defective in von Gierke's disease) and found it to be present in amounts within the normal range :

- (a) phosphorylase b
- (b) glucokinase
- (c) glucose 6-phosphatase
- (d) amylo 1, 6 glucosidase

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2. The fact that the response to adrenalin was negative suggested that any one of several enzymes might be absent or defective. One of the following groups lists the enzymes which have in common the feature that a defect in any one of the enzymes would lead to the negative adrenalin response. Choose that group:

- (a) 3', 5'-AMP-cyclase, phosphorylase b kinase, amylo-1, 6 glucosidase, phosphorylase a and phosphoglucomutase
  - (b) glucose-6-phosphate dehydrogenase, amylase, phosphohexoseisomerase and aldolase
  - (c) UDPG-glycogen synthetase, phosphorylase a, phosphoglucomutase transaldolase and transketolase
  - (d) amylo-1, 4  $\rightarrow$  1, 6 transglucosylase, amylo-1, 6 glucosidase, phosphorylase a and UDPG-glycogen synthetase.
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TABLE FOR QUESTIONS 1 —> 7

Disease	Glycogen Level	Blood Sugar Level	Sugar Tolerance*	Response to Adrenalin	Glycogen Structure
von Gierke's	Markedly elevated in liver but normal in muscle	Very low	Normal to glucose; abnormal to galactose and fructose	No rise in Blood sugar level	Normal
Present patient	Markedly elevated in liver but normal in muscle	Normal to slightly low	Normal to glucose, galactose and fructose	No rise in blood sugar level	Not done

\* Rate of disappearance of injected or ingested sugar from circulating blood.

3. Again he found that each of the enzymes tested was present in essentially normal amounts. As a result of these findings, Mr. Harbandray felt that some clue to identifying the defect would emerge from an examination of the glycogen structure. He knew that normal glycogen incubated with purified phosphorylase a, phosphate and amylo-1,6 glucosidase should be degraded completely to :

- (a) a mixture of glucose-1-phosphate and sucrose
  - (b) " " " " " " " glucose 6-phosphate
  - (c) " " " " " " " glucose
  - (d) " " " " " " " maltose
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4. He observed, however, that in the test performed above the glycogen isolated from the liver biopsy yielded only glucose-1-phosphate. Since the amount of glucose-1-phosphate formed was not very large but rather about what one would have expected from the action of phosphorylase a alone on normal liver glycogen, he concluded that the glycogen contained :

- (a) excessively short outer chains
  - (b) excessively long outer chains
  - (c) normal outer chains
  - (d) nothing but  $\alpha$  1,6 glucosyl linkages
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5. From his experiments thus far Mr. Harbandray reasoned that there was a defect in the glycogen structure and not in the enzymes which degrade it. Moreover, he realized that the aberrant structure must result from a defect in one of the enzymes involved in glycogen synthesis. He argued that the defect was probably not in UDPG-glycogen synthetase. He recalled, moreover, that a loss of amylo-1,4  $\rightarrow$  1,6 transglucosylase activity would result in the formation of :

- (a) a glycogen with a greater than normal proportion of branches
  - (b) a glycogen with a normal number of branches
  - (c) a glycogen with no branches
  - (d) a glycogen containing only  $\alpha$ -1,6 glucosyl linkages
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6. Even though the existence of such a defect was not wholly consistent with his finding in #4, he decided to go ahead and assay the extracts from the patient's liver biopsy for amylo-1,4  $\rightarrow$  1,6 transglucosylase activity in the following way. The  $C^{14}$ -labeled glycogen substrate used in the assay was prepared from  $C^{14}$ -glucose-1-phosphate, unlabeled glycogen as primer, and phosphorylase a. Upon incubating this labeled glycogen with normal amylo-1,4  $\rightarrow$  1,6 transglucosylase he expected that subsequent exposure of the product to phosphorylase a and phosphate would remove :

- (a) all the  $C^{14}$ -glucose residues as glucose-1-phosphate
  - (b) only a portion of the  $C^{14}$ -glucose residues but these would all be glucose-1-phosphate
  - (c) part of the  $C^{14}$  as glucose-1-phosphate and the remainder as glucose
  - (d) nothing
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7. When he assayed extracts from the patient's liver by this technique, much to his surprise he found essentially normal levels of transglucosylase activity. He was faced therefore with the dilemma of rationalizing how in the presence of normal amylo-1, 4  $\rightarrow$  1, 6 transglucosylase, a glycogen was formed which could not be split by the combined action of phosphorylase and amylo-1, 6 glucosidase. Realizing that something might be strange about the inter-glucosyl linkages being formed with the patient's enzyme, he asked one of his graduate student friends in the chemistry department to determine the type of linkages between glucose residues in a sample of the infant's liver glycogen. Back came the answer that the predominant linkages were 1, 4, some were 1, 3, but no 1, 6 linkages. Mr. Harbandray saw the answer immediately; the defect in glycogen structure which prevented it from being metabolized was due to:

- (a) a modified UDPG-synthetase which formed 1, 3 instead of 1, 4 linkages
  - (b) an altered amylo-1, 6 glucosidase which could split 1, 3 linkages
  - (c) the suspected amylo 1, 4  $\rightarrow$  1, 6 transglucosylase which was in reality an amylo 1, 4  $\rightarrow$  1, 3 transglucosylase
  - (d) a modified phosphorylase a which could not phosphorylyze 1, 3 linkages
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For his ingenious solution to this case Mr. Harbandray was awarded the John A. Berg research medal and this disease came to be known as Harbandray's syndrome.

8. Succinyl CoA has been found to undergo all of the following enzymatic transformations except :

- (a) hydrolytic deacylation
  - (b) CoA transfer to acetoacetate
  - (c) isomerization to form methyl malonyl CoA
  - (d) fixation of CO<sub>2</sub> to form  $\alpha$  ketoglutaryl CoA
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9. When ATP, CoA, stearic acid and Mg<sup>++</sup> are incubated with the enzyme stearyl thiokinase, stearyl CoA, AMP and pyrophosphate are formed in equimolar amounts. One proposal is that stearyl adenylate is an intermediate in this reaction. Which of the following experimental observations supports the proposed role of stearyl adenylate as an intermediate :

- (a) the radioactivity of P<sup>32</sup>-labeled pyrophosphate will exchange with ATP in the presence of enzyme, Mg<sup>++</sup> and stearate,
  - (b) the radioactivity of P<sup>32</sup>-labeled pyrophosphate will exchange with ATP in the presence of enzyme, Mg<sup>++</sup> and CoA,
  - (c) C<sup>14</sup>-labeled AMP will exchange with ATP in the presence of enzyme, Mg<sup>++</sup> and stearate,
  - (d) C<sup>14</sup>-labeled AMP will exchange with ATP in the presence of enzyme, Mg<sup>++</sup> and CoA,
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